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Agricultural Research Institute, Pusa

The Pebrine Disease of Silkworms in India

BY

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The Pebrine Disease of Silkworms in India.

[Received for publication on 1st May, 1917.]

Professor Lefroy in the course of his enquiry into the conditions responsible for the decadence of the Indian Silk Industry, came to the conclusion that one of the principal causes of its present condition is the excessive prevalence of "*Pebrine*," the disease of silkworms which wellnigh extinguished the industry in Europe, in the middle of the nineteenth century. As is well known, Pasteur, after two years' work, demonstrated that the cause of this disease was a protozoon parasite and devised a method of checking its spread and reducing its occurrence to comparatively negligible proportions; this method has now been successfully used in France and Italy for nearly forty years, and was introduced into India some fifteen years since, where it has been adopted in the Government seed-rearing nurseries in Bengal and elsewhere. Professor Lefroy's inquiries showed that as a means of eliminating pebrine the Pasteur method as used in Bengal has been a failure, and it therefore became imperative to determine the reasons for its defective operation in order to discover whether the method itself was inapplicable in this country on account of the difference between European and Indian conditions, or whether some modification of it could be devised which would render it as effective in the East as it has been found in the West. At Professor Lefroy's suggestion I commenced an enquiry into the subject, primarily with a view to determining why the Pasteur method had failed in Bengal and if possible to discover a suitable modification or alternative. This Bulletin is intended as an *interim* report of my investigations to date and is published in order to describe and recommend the trial of a modification of the Pasteur method which I have devised as a result of my enquiries and which has been found successful on a small scale at Pusa.

The Pasteur method. Pasteur's researches resulted in the discovery that certain microscopic bodies ("corpuscules") which some earlier workers had considered to be normal constituents of the body fluids of the silkworm, were in fact parasites responsible for the disease and carried it from one generation to the next through the eggs of the moth. Nearly every diseased moth produces diseased eggs and although every egg in an infected laying is not necessarily diseased yet the percentage is

high enough to ensure serious mortality amongst the worms. In addition to this hereditary infection the disease is also spread by contamination of the food by the droppings of diseased worms which contain pebrine spores, the infection in this case being carried on by ingestion of these spores with the food of the healthy worms. In this way an infected brood or "laying" will spread infection to otherwise healthy worms, so that hereditary infection of a small percentage will result in rapid spread of the disease, and on the other hand if eliminated should go far to extinguish it completely. That such extinction has not been obtained after forty years' use of the method in Europe seems to suggest that as applied in practice it does not afford a complete protection from hereditary transmission even in Europe.

The method itself depends upon the fact that the "Pebrine bodies," the spore form of the parasite, are readily recognizable under a comparatively low power of the microscope, thus making it possible to detect their presence without the use either of expensive apparatus or elaborate technique, in fact this is habitually done by entirely unskilled native rearers in Bengal. This recognizability is due partly to the comparatively large size and characteristic oval shape of the pebrine bodies and partly to their highly refringent character, which makes them clearly distinguishable in the water in which examination takes place from other bodies of similar dimensions which may be present. The body of the moth is crushed with a little water, a drop of the resultant fluid is placed on a slide and examined under a magnification of some 500—600 diameters; if pebrine bodies are seen the eggs laid by this moth are destroyed, otherwise they are passed as disease-free. Now it will be evident that **the success of this method as a means of eliminating hereditary infection depends upon the general assumption that if there is sufficient disease present in a moth to affect its progeny, its presence will be detectable in the above described manner**; this is the assumption upon which the successful use of the Pasteur method in Europe depends, but my inquiries appear to show that **so far as India is concerned the method requires serious modification** for the following reasons.

The essential difference between European and Indian conditions, so far as the life history of the mulberry silkworm is concerned, lies in the fact that in Europe the worm is univoltine, *i.e.*, produces only one generation in the year whereas in India the races are in most cases multivoltine, producing seven or eight generations. As a corollary of this difference the eggs of the multivoltine races hatch out within some eight days after laying, whereas in Europe the univoltine eggs are laid in the summer and do not hatch until the following spring. The practical

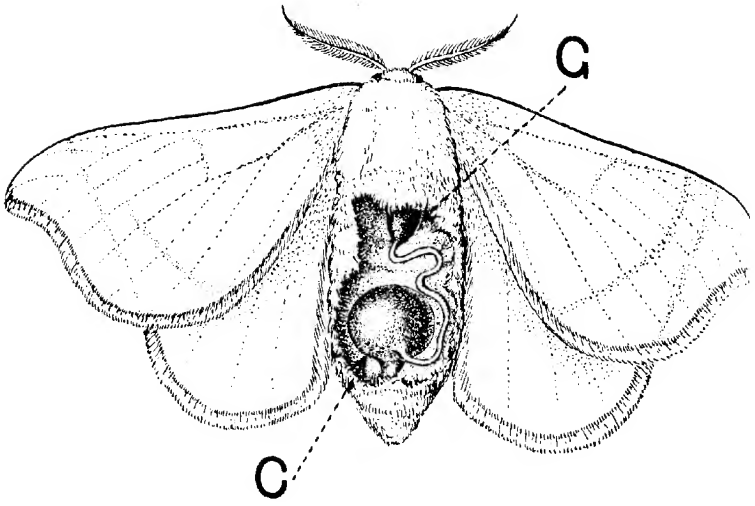


Fig. 1.

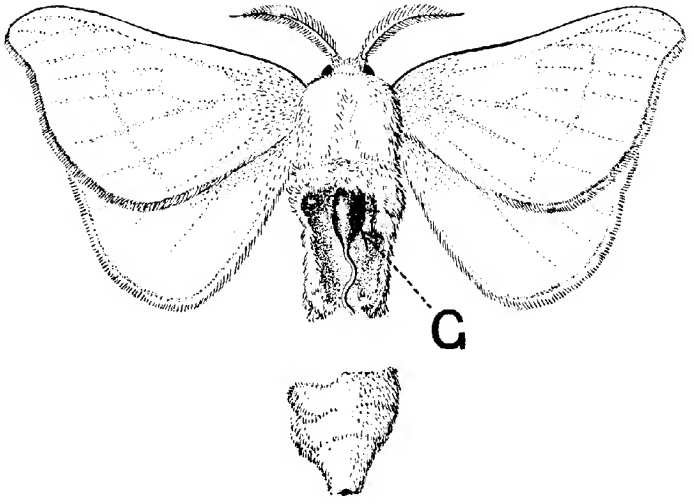


Fig. 2.
Gut (G) and Colon (C) of Moth.

effect of this difference is to make it necessary in India to carry out the examination of the parent moth within the comparatively limited period of one week from the date of laying, whereas in Europe this period can be, and generally is, extended for convenience sake to some weeks or months. Now it has been found in India that a considerable percentage of worms derived from moths examined and passed as disease-free develop pebrine notwithstanding such examination, and the first conclusion arrived at was that the examination was defective because of the assumption that if disease was present it would be found by the very rough method in vogue ; this method practically assumes that the pebrine bodies if present at all will be so universally distributed throughout the body of the moth that some of them are certain to be found in the minute fraction of the whole represented by the field of view of the microscope. Investigation showed that under the conditions obtaining in India this assumption was unwarranted ; numerous cases occurred in which no pebrine bodies were found by the ordinary method of examination whereas they were readily detectable by the modified one which I wish to recommend ; moreover the disease showed itself in the progeny although the parent moths would have been passed as disease-free by any user of the routine method. The Indian method of examination also differs from the European not only in point of time elapsing between laying and examination, but also in the method of taking the material for examination. In Europe the body of the moth when examined some months after death is perfectly dry, and water is added to the crushed tissues for examination. In India the fresh body of the moth contains body fluids of various kinds and the technique usually adopted, simply crushing the body and smearing the exudate upon a slide, results in the major portion of the fluid examined being derived from a single organ, the Colon (Plate I, Fig. 1 C), so that the result of the examination depends largely upon the invasion of the lumen of this part of the a imentary tract by the parasite. I have found by examination of several hundreds of specimens that pebrine bodies may be almost absent from the liquid from this organ although present in the ovaries and other parts of the body, and this fact would account for the failure of this method of examination to detect the disease.

By examination of large numbers of pebrinized moths it was found possible to ascertain in what part of the body the pebrine corpuscles first seem to appear, or at least in what part they are invariably found if any are found at all. This part of the moth is the gut or chyle stomach, readily accessible by mere separation of the lower portion of the abdomen, with a needle, forceps, or other instrument leaving the dark canal of the gut exposed as shown in

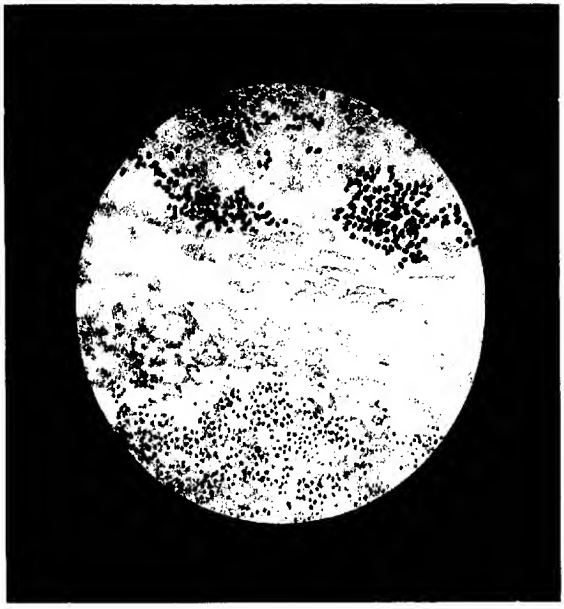
Plate I, Fig. 2 G. A minute portion of the gut removed on the needle and rubbed with water on the slide will show the presence of pebrine bodies if they occur in the body of the moth in sufficient number to be detected by rough microscopic examination.

It may be of interest here to give the probable reasons why the presence of pebrine is more likely to be detected by examination of the tissues of the gut than of any other part. Infection, except hereditary infection, takes place through the food and so through the alimentary canal and particularly in the gut; the infecting bodies find their way from the gut through its walls into the cells (epithelial) which form the lining layer of the gut and whose function is to secrete digestive fluids. In these cells then actual invasion of the tissues of the host first takes place and from them the infection spreads by movement and multiplication of the invading parasites; it follows that as the parasite grows by feeding on the tissues of its host the food supply afforded by the latter must fail earliest at that point which was first invaded, and as failure of nutriment results in the passage of the protozoon into the resting or spore condition, and it is in this condition that the parasite is readily recognizable under microscopic examination, it will readily be understood why the tissues of the gut, where infection first took place, present the most likely point for discovering its presence, and although the gut in the moth is practically functionless it is known that the elements from which it is built up in the reconstructive process during the pupal stage are identical with those of the larva from which they are derived.

Now in the case of dried bodies of moths examined some weeks after death the condition of affairs appears to be different, for in no case were pebrine corpuscles found in the gut and not in other organs; in fact it may be said that when pebrine was found in any part of the dried body it was discoverable in practically every part; the apparent conclusion would be that the pebrine parasite continued to multiply in the body of the moth after death until the whole of the tissues were invaded; in view of the rapid drying up of the latter this seemed a difficult theory to accept and further investigation has now led me to advance an alternative one.

The life cycle of the pebrine parasite, *Nosema bombycis*, as worked out by Stempel, and of other similar Microsporidia such as *Nosema apis* (Fantham and Porter) and *Nosema culicis* (Korke) includes transition through an actively multiplying stage (merogony) to the resting or spore stage (sporogony).

During the first stage the organism would be recognizable only with difficulty under the microscope under the conditions in which examination is ordinarily carried out; in the second or spore stage it is readily



Showing (small) active multiplying forms and (large) resting spores, in gut wall of moth.

recognized by reason of the thick refractile envelope or sporocyst characteristic of the resting condition. Now the function of the spore is to carry the parasite through conditions unfavourable to its active life, such conditions including absence of suitable nourishment and of moisture; when the supply of food for the parasite is exhausted by the penetration of the latter to all the tissues of the host, or by the drying up of the dead body of the moth, the protozoon passes into the resting or spore stage simultaneously becoming recognizable under ordinary examination. (Plate II.) For this reason a moth which, although full of pebrine, might be passed as disease-free by the routine method of examination a few days after laying, would, if kept for some weeks, appear obviously pebrinized. This fact will explain the greatly increased probability of detecting pebrine by the Pasteur method in Europe working with univoltine races as compared with the chance of doing so with the method as used in Bengal with multivoltine varieties. At the same time it must be pointed out that even in Europe the detection of pebrine depends upon the presence in the moth of a sufficiently large number of parasites to ensure the presence of some of them in the field of the microscope, and there can be no doubt that the failure of the Pasteur method to eliminate the disease after 40 years' trial must be largely due to the escape of those individual cases in which sufficient parasites were present to infect the ovaries and consequently the eggs, but not to allow of detection by the routine method of examination. In Europe, however, the percentage affected is so small that no demand appears to exist for any improvement in the method, and in fact the use of the so called "industrial" seed derived from moths of which only a certain percentage have been examined, is evidence of the absence of such a demand. Should conditions ever require more stringent control this could be effected by the use of the more particular method of examination which I have described above, the adoption of which I consider necessary in India for the selection of disease-free seed in the rearing of multivoltine races of the mulberry silkworm.

PUSA,

30th April, 1917.

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